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Discrepancy in fine root turnover estimates between diameter-based and branch-order-based approaches: a case study in two temperate tree species

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Abstract: Fine root turnover plays a key role in carbon (C) budgets and nutrients cycles in forest ecosystems. However, the difference between branch-order-based and diameter-based approaches in estimating fine root turnover is still unclear. We studied root biomass turnover based on multiplying root standing biomass by turnover rate (inverse of median root longevity) in two Chinese temperate tree species, *Fraxinus mandshurica* Rupr. and *Larix gmelinii* Rupr. The minirhizotron (MR) technique was used to estimate longevities for first and second order roots, and total roots (R_{total}) apparent on the MR tube surface. The corresponding biomass for each root group was estimated by soil monolith. The difference in biomass turnover between R_{total} and the sum of the first and second order roots was used to represent the discrepancy between diameter- and order-based approaches. First order roots had shorter life spans and higher biomass turnover rates than the second order roots in both species. Biomass turnover estimated by the order-based method for *F. mandshurica* and *L. gmelinii* were 155.4 g·m^{-2}·a^{-1} and 158.9 g·m^{-2}·a^{-1}, respectively, in comparison with 99.5 g·m^{-2}·a^{-1} and 117.7 g·m^{-2}·a^{-1} estimated by the diameter-based method, indicating that the diameter-based approach underestimated biomass turnover. The most probable reason was that the order-based method enhanced separation of the heterogeneous root population into relatively homogenous root groups with varying turnover rates. We conclude that separating fine root pool into different branch orders can improve the accuracy of estimates for fine root turnover, as well as the understanding of the belowground C allocation and nutrient cycling at ecosystem level.

Keywords: *Fraxinus mandshurica*; root life span; root longevity; *Larix gmelinii*; minirhizotron; soil monolith

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Introduction

Fine roots are the most dynamic portion of the tree root system (Pregitzer 2002; Eissenstat and Yanai 2002), and they play a key role in carbon (C) budgets and nutrients cycles via turnover at the ecosystem scale (Jackson et al. 1997; Norby and Jackson 2000). However, estimates of fine root turnover time (i.e., longevity) range from a few months to several years among species and across ecosystems (Burton et al. 2000; Withington et al. 2006; Peek 2007), and large discrepancies may lead to uncertainty in estimating ecosystem C flows and nutrients cycles (Majdi et al. 2005; Guo et al. 2008a). Discrepancies might be related to variations in climate (Tierney et al. 2003) or soil (Burton et al. 2000), or might be species specific (Withington et al. 2006). However, conceptual and methodological approaches might also affect estimates of fine root turnover (Hendricks et al. 2006; Guo et al. 2008a).

Fine roots have traditionally been treated as a homogeneous pool, assuming that all roots of a given diameter size have the same function, production and mortality (Pregitzer et al. 2002). This assumption was the basis of many studies that used the minirhizotron (MR) technique to estimate fine root longevity and turnover rate (Johnson et al. 2001). In the last decade, however, some studies revealed that trees roots with a given narrower diameter size (e.g. <0.5 mm) were highly heterogeneous, had complex branching structures, and consisted of numerous individual roots differing in morphology (Pregitzer et al. 2002), anatomy (Guo et al. 2008c), chemistry (Pregitzer et al. 2002) and physiology and longevity (Pregitzer et al. 1998; Majdi et al. 2001; Wells et al. 2002; Guo et al. 2008b). The difference in longevity of roots of different branch orders was ignored when using the traditional diameter-based method, and this might have caused uncertainty in estimating biomass turnover. A simulating model study suggested that the diameter-based method would increase the error of estimated turnover rate, but this error could

be greatly reduced if the roots were sorted by branch order (Guo et al. 2008a). Therefore, partitioning of the root population by branch order would be expected to increase the precision of estimates of root longevity and turnover (Trumbore and Gaudinski 2003; Guo et al. 2008a).

Determining fine root turnover based on branch order requires estimation of both root longevity and standing biomass (Majdi et al. 2005). Traditional sequential soil cores had the advantage in estimating standing biomass, but failed to concurrently estimate production and mortality (Vogt et al. 1998; Hendricks et al. 2006). In contrast, the MR method enables definition of the demography of the various branch orders (Wells et al. 2002; Guo et al. 2008b) or diameters of roots (Wells and Eissenstat 2001; Baddeley and Watson 2005), but does not yield direct estimates of biomass (Johnson et al. 2001). Some studies transformed root length data to biomass by linking root diameter (from MR) to specific root length (SRL, from soil core) (Steele et al. 1997; Steinaker and Wilson 2005; Iverson et al. 2008), or root length density (from MR) to standing biomass (from soil core or soil monolith) (Hendrick and Pregitzer 1993; Hendricks et al. 2006). However, the small viewing area of the MR camera limited our ability to determine the order for individual roots (e.g. Wells et al. 2002), and this hindered transforming all root lengths of each order to biomass. Thus, combining the MR method which estimates root longevity of different orders, with sequential soil cores (or soil monolith), which estimates the corresponding standing biomass, enabled calculation of fine root biomass turnover on a root order basis (Majdi et al. 2005; Guo et al. 2008b). However, few studies investigated fine root biomass turnover based on branch order, and also determined the difference between order-based and diameter-based approaches.

We conducted a two-year study using the MR method in two Chinese temperate species, *Fraxinus mandshurica* Rupr. and *Larix gmelinii* Rupr., to estimate root longevity. We estimated the standing biomass of roots of various orders by sampling soil monoliths to depths of 0–20 cm. Previous studies reported that the greater proportion of roots appearing on the MR tube surface was accounted for by first order roots in peach trees (90%–93%, Wells et al. 2002) or by first and second order roots in longleaf pine (*c*. 95%, Guo et al. 2008b). We assumed that our two species followed this pattern and that all roots appearing on our MR tubes represented a naturally mixed population of roots of the first two orders (here abbreviated as R_{total}). In the diameter-based approach, root turnover rate (inverse of median longevity) was estimated directly by R_{total} via MR measurement, regardless of order rank for individual roots. The corresponding standing biomass could be estimated indirectly as the sum of the biomass of the first and second order roots yielded by soil monolith. In the branch-order approach, turnover rate and corresponding standing biomass for the first and second order roots were estimated directly by MR and soil monolith, respectively. Our overall objective of this study was to quantify differences in root biomass turnover estimates produced by the order-based versus the diameter-based approaches, and to assess the causes of any differences.

Materials and methods

Study site and plot establishment

This study was conducted at Maoershan Forest Research Station (45°21'–45°25' N, 127°30'–127°34' E) of Northeast Forestry University, in Heilongjiang Province, China. The study area has a continental temperate monsoon climate with mean January, July and annual temperatures of -19.6°C, 20.9°C and 2.8°C, respectively, and a growing season length ranging from 120 to 140 days. Mean annual precipitation is 723 mm with 477 mm distributed in June, July and August (Zhou 1994). Soils are Hap-Boric Luvisols (Gong et al. 1999) that exceeds 50 cm in depth and with high organic matter content, have well-developed horizons, and are well drained. Other site properties and soil characteristics were reported by Wang et al. (2006).

Two plantations, *F. mandshurica* and *L. gmelinii*, were selected on a southwest-facing slope of approximate 13°, at elevation ranging from 450 m to 500 m. Both plantations were established in 1986 by planting two-year-old seedlings in a 1.5 m × 2.0 m planting grid. In 2003, *F. mandshurica* and *L. gmelinii* trees had trunk diameters averaging 9.1 cm and 11.7 cm at breast height, respectively. Prior to root sampling in May 2002, three 20 m× 30 m sampling plots were sited randomly in each plantation.

Minirhizotron assessments of root longevity and morphology

Six MR tubes (90 cm length, 5.1 cm inner diameter) were installed randomly in each plot in October 2003 with a total of 18 tubes for each species. The tubes were installed at an angle of 45° to the soil surface, and a maximum observation depth of approximately 40 cm, which represented approximately 95% of the soil depth and 98% of root biomass at the site (Cheng et al. 2005; Mei et al. 2006). Tubes were sealed with a rubber stopper and wrapped in black tape to keep light and rain from entering. The distance between an MR tube and any trunk was at least 0.5 m. From 16 April 2004 to 25 October 2005, video images were collected at about two week intervals using a micro-video camera (BTC-2, Bartz Technology, Santa Barbara, CA) during the growing season. The six-month time lag between installation and observation was to allow for re-equilibration following disturbance and to improve contact between the soil and tube surface (Baddeley and Watson 2005). A previous study at the same site and also using the MR method showed that root length and mortality more closely approximated production in the two study species during the growing season, indicating the re-equilibration of root dynamics at the tube surface was reached (Shi et al. 2007). Video images were digitized via RooTracker software (Duke University Phytotron, Durham, NC). Data recorded for each root include of the date of appearance, date of death (apparent blackening or shriveling), date of disappearance, soil depth, diameter and root order. Root branch order was designated according to Wells et al. (2002). Specifically, roots with zero, one or two or-

ders of visible dependent laterals categorized as first, second or third order roots, respectively. Due to the limited viewing area of the minirhizotron camera, the definition of root order might have been biased to a degree, but the high branching ratio between the first and second order roots of the two study species largely reduced such bias (Wang et al. 2006; Guo et al. 2008b). Only those roots emerging in 2004 were tracked and used in subsequent analyses. Overall, total numbers of 609 and 229 roots of *F. mandshurica* and *L. gmelinii* were observed at 0–40 cm soil depth, and 536 and 207 roots were categorized to order rank for *F. mandshurica* and *L. gmelinii*, respectively. Roots of questionable order were considered most likely to be of the first two orders according to Wells et al. (2002) and Guo et al. (2008b). Root longevities and turnover rates were both estimated for total roots (diameter-based) and each order of roots (branch order-based).

Root morphology and standing biomass assessment by soil monolith

Three random monoliths (20 cm × 20 cm × 10 cm) from each plot were taken at two continuous soil depths (0–10 cm and 10–20 cm) in May, July, and September in 2003. Thus, a total of 18 soil blocks were excavated for each species. Once excavated, the intact soil blocks were put into a cooler with ice and transported to the laboratory within several hours and frozen for subsequent root processing. The soil attached to an intact root branch was brushed away carefully, and then some small segments were washed with deionized water (1°C) to remove soil particles. Following Pregitzer et al. (2002) and Guo et al. (2004), all cleaned root segments were then dissected into different branch orders under a 10 × stereomicroscope. A maximum category of fifth order roots was included in subsequent morphology and biomass analyses. Because of the large number of roots in each soil block sampled by monolith, we randomly selected 500, 80, 40, 20, 10 roots of the first five orders respectively for morphology analyses. Root diameter and length of each root were measured to the nearest 0.05 mm under 20 × magnification with an ocular micrometer fitted on the stereomicroscope. In September, we excavated one additional soil block in one of three plots for each species, to determine the frequency distribution of root diameters, regardless of their order ranks. This sampling was motivated by the need to assess whether the dynamics shown by MR reflected the behavior of roots in bulk soil. Living and dead roots were classified by figuration, color and flexibility (Hendricks et al. 2006). In each soil block, all roots of a given order were dissected and then oven dried to constant weight (65°C), then weighed (nearest= 0.0001 g). Root subsamples were placed in a muffle furnace (550°C for 5 h) to determine ash content. Root biomass estimates per unit area (g·m^{-2}) were expressed on an ash-free, dry mass basis. The standing biomass of first and second order roots was used to estimate biomass turnover.

Data analysis

Mean and median root longevities for each order and R_{total} were

both analyzed using Kaplan-Meier survival analysis (Lee & Wang 2003) provided by the procedure Survival in SPSS (2004, v.13.0; SPSS Inc., USA). Root longevity was defined as days between initial appearance and death. Roots living past the last observation were considered right censored; i.e., survival time was at least as long as the time to final observation. Winter is long in our region (*c.* six months), and root mortality in winter is low (Yu et al. 2007). As suggested by Andersson and Majdi (2005), disregarding the duration of winter in survival analysis might be more reasonable for the estimate of root longevity, so we ignored the winter season of 192 days in this study. Winter was defined to extend from the last observation date in 2004 to the first sampling effort in 2005. An inverse of median root longevity estimated by survival analysis was calculated as the turnover rate. The product of annual standing biomass (from soil monoliths) and turnover rate was defined as root biomass turnover (Majdi et al. 2005; Guo et al. 2008b). The difference in biomass turnover between diameter-based and order-based approaches was assessed by comparing the sum of turnovers in the first and second orders with R_{total} turnover.

Results

Roots from *F. mandshurica* and *L. gmelinii* obtained by either MR or soil monolith, exhibited large variation in diameter, ranging from less than 0.1 mm to >2.0 mm (Fig. 1). The frequency distributions of diameter classes obtained by the two methods were similar for the two species (Fig. 1), indicating that root behavior on the MR tube surface was consistent with that in bulk soil. On MR tubes, more than 90% and 50% of the first and second order roots were <0.5 mm in diameter in both species, but 81% (*F. mandshurica*) and 62% (*L. gmelinii*) of R_{total} were <0.5 mm in diameter (Fig. 1). In soil monoliths, over 95% and 80% of first and second order roots were <0.5 mm, however, R_{total} reached 90% in both species (Fig. 1).

Standing biomass of first and second order roots in both species was relatively stable during the growing season, although that of the first order roots from *L. gmelinii* was much larger in September (Fig. 2). Standing biomass of first order roots was consistently larger than for second order roots. Mean annual standing biomass of R_{total} from soil monoliths was 98.4 g·m^{-2} for *F. mandshurica* and 51.0 g·m^{-2} for *L. gmelinii*, respectively, in which 70% (*F. mandshurica*) and 60% (*L. gmelinii*) were accounted for by first order roots.

Median and mean longevity were shortest for first order roots, longest for second order roots, and intermediate for R_{total} (Table 1). Turnover rate during the growing season (180 days) thus showed an inverse pattern, with first order roots having most rapid turnover while second order roots had the shortest turnover rates in both species (Table 1). However, survival curves of R_{total} for both species more closely approximated those of first order roots (Fig. 3). Calculating from standing biomass (Fig. 2) and turnover rate (Table 1), on an order-based method, biomass turnover for the first and second order roots combined was 155.4 g·m^{-2}·a^{-1} for *F. mandshurica*, which was 56.2% higher than that

of R_{total} (99.5 g·m^{-2}·a^{-1}) as estimated by the diameter-based method (Table 1). For *L. gmelinii*, biomass turnover estimated by the order-based method was 159.1 g·m^{-2}·a^{-1}, which was 35.0%

higher than that of R_{total} (117.7 g·m^{-2}·a^{-1}). In addition, biomass turnover of first order roots was markedly greater than for second order roots across species (Table 1).
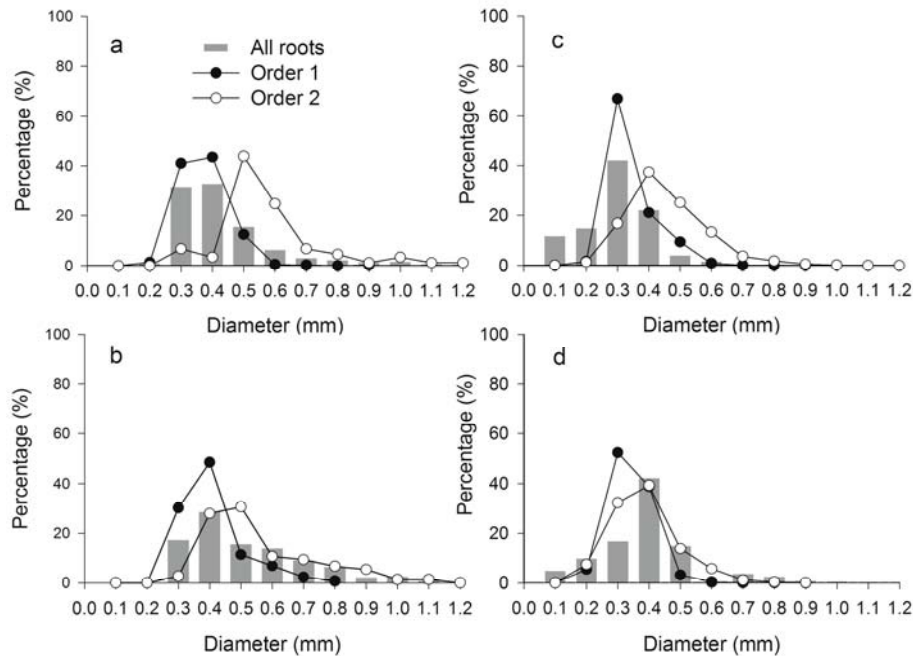

Fig. 1 Percentage frequency distribution of root diameter in 0.1 mm classes for first and second order roots, and all roots. Fig.1a and c represent the frequency distributions of roots of *Fraxinus mandshurica* observed by minirhizotron and sampled by soil monolith, respectively. Fig.1b and d represent the frequency distributions of roots of *Larix gmelinii* observed by minirhizotron and sampled by soil monolith, respectively.
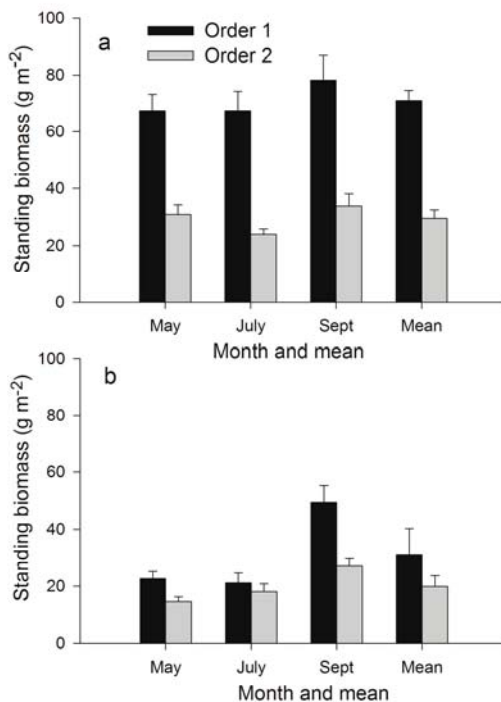

Fig. 2 Standing biomass (± SE) in three months and annual mean for first and second order roots sampled by soil monolith at 0-20 cm soil depth. (a) *Fraxinus mandshurica*; (b) *Larix gmelinii*.
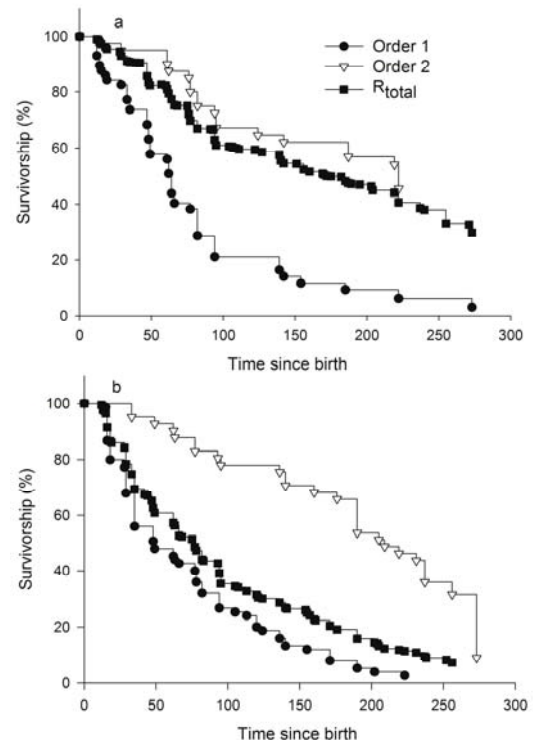

Fig. 3 Survival probabilities for first, second order roots and R_{total} during 2004-2005. (a) *Fraxinus mandshurica*; (b) *Larix gmelinii*.

Table 1. Root longevity, turnover rate, biomass turnover and other root characteristics for first, second order roots and all roots (R_{total}) from _Fraxinus mandshcurica_ and _Larix gmelinii_ during the growing season.

Species, root category	Number	Diameter (mm)	Median longevity (day)	Mean longevity (day)	Turnover Rate (growing·season^{-1})[*]	Mortality rate (%)	Biomass turnover (g·m^{-2}·a^{-1})
F. mandschurica							
First order	447	0.32(0.01)	95(10)	132(4)	1.89	79.2%	134.5
Second order	89	0.56(0.03)	255(8)	230(8)	0.71	52.8%	20.9
R_{total}	609	0.43(0.01)	182(14)	175(4)	0.99	67.2%	99.5
L. gmelinii							
First order	132	0.35(0.01)	42(3)	60(4)	4.29	98.5%	133.3
Second order	75	0.68(0.03)	140(38)	158(10)	1.29	80.0%	25.6
R_{total}	229	0.48(0.01)	78(8)	105(6)	2.31	92.1%	117.7

[*] Turnover rate = growing season days / median longevity. Values in parentheses were standard error (SE), and growing season was 180 days.

Discussion

During past decades, fine root production and mortality have been widely studied using the MR method (Hendrick and Pregitzer 1993; Burton et al. 2000; King et al. 2002; Majdi et al. 2005), yet few investigations have applied the branch order method (but see Guo et al. 2008b) and compared these results with those from diameter-based approaches. One possible reason is the difficulty of sampling roots of different orders from soil and then estimating standing biomass per unit area. Recent studies have determined root morphology and tissue chemistry of different branch orders by studying intact soil cores or monoliths (Pregitzer et al. 2002), and have estimated standing biomass at the ecosystem level (Guo et al. 2004; Wang et al. 2006). These studies are relevant to the present research. Another important question is whether roots appearing on the surface of MR tubes could be similar to those growing in bulk soil. Our study showed that the frequency distribution of root diameters under both conditions were quite similar in our two study species (Fig. 1), consistent with the findings by Withington et al. (2006). In addition, at our study site, Mei (2006) reported that fine root standing biomass (<1 mm in diameter at 0–20 cm depth) estimated by sequential soil cores was 108.7 g·m^{-2} (_F. mandshurica_) and 68.6 g·m^{-2} (_L. gmelinii_), slightly greater than 98.4 g·m^{-2} (_F. mandshurica_) and 51.0 g·m^{-2} (_L. gmelinii_) for the first two order roots estimated by soil monolith in this study (Fig. 2). This result is possibly because the fine root pool (<1 mm diameter) certainly contained a portion of higher order roots (such as third and fourth orders, see Wang et al. 2006), but most were roots of the first two orders. These results suggest that combining MR with soil monolith can properly estimate root turnover for the various branch orders.

Compared with the branch order-based method, the traditional diameter-based approach (R_{total}) generally underestimated biomass turnover for both species (Table 1), ranging from 35.0% (_L. gmelinii_) to 56.2% (_F. mandshurica_). Several reasons might account for this result. R_{total} was a mixture of a heterogeneous 'root population', containing both a short-lived group (i.e., first-order roots) and a relative long-lived group (i.e., second-order roots), thus turnover rate and biomass turnover should be characterized separately for these two groups (Joslin et al. 2006). In other words, fine root turnover rate, as well as the corresponding biomass turnover, would depend on the relative proportion of short-lived and long-lived roots. For the two species in this study, the number of first-order roots accounted for at least 73% (_F. mandshurica_) and 58% (_L. gmelinii_) of total root numbers, while their standing biomass and turnover accounted for 71% and 87% for _F. mandshurica_, 65% and 84% for _L. gmelinii_, respectively. However, biomass turnover estimated by the diameter-based method (R_{total}) could not represent the most dynamic part, i.e., the first order roots, resulting in a lower estimate than the branch-order-based approach (Table 1). Our results empirically proved that fine root biomass turnover estimated by the branch order method is more effective than the diameter method, as theoretically predicted by Guo et al. (2008a). As the first order roots generally accounted for a larger proportion of root numbers and biomass per unit area in some temperate and subtropical tree species (Pregitzer et al. 2002; Wells et al. 2002; Guo et al. 2004; Wang et al. 2006), underestimation of turnover by the diameter-based approach might be common. It should be noted that fine root pool in this study was limited to the first two root orders. If the diameter size threshold was increased in a diameter-based method, then some higher order roots would be included into the fine root pool. If so, what would be the difference in production estimates between the two methods? We speculate that an underestimate of biomass turnover would also result. Because standing biomass of first order roots is as much or more than that for third and fourth order roots in both species in our previous study (Wang et al. 2006), and the higher order roots have much lower turnover rates (longer life span, Espeleta et al. 2009). In addition, an anatomical study based on branch order with 23 temperate tree species (including our two species) by Guo et al. (2008c) proved that the first order roots generally have primary structure, and parts of the second or third order roots have secondary development, while the fourth and fifth order roots mainly serve a transport function. Their study indicated that the first two order

roots may generally be non-woody, and should have shorter life spans in comparison with much higher orders. In this manner, the difference in biomass production in the first two branch orders between branch-order and diameter-based methods may well represent the discrepancy in the whole root population between different methods.

First order roots had consistently greater biomass turnover than did second order roots, due to their greater standing biomass and shorter life span (Table 1). Increase in root life span with ascending branch order is also confirmed for some conifer species (e.g., *Picea abies*, Majdi et al. 2001; *Pinus palustris*, Guo et al. 2008b), hardwoods (*Acer saccharum*, Wells 1999) and fruit trees (*Prunus persica*, Wells et al. 2002). Lower order roots definitely develop from the stele of higher orders, so death of a higher order root must lead to death of the affiliated lower order roots (Majdi et al. 2001). Guo et al. (2008c) also found that first order roots are comprised primarily of cortical cells and tend to be damaged or lost under stress (Wells and Eissenstat 2003), and to exhibit shorter life spans (Wells et al. 2002; Guo et al. 2008b and this study). Higher standing biomass for first order roots might be related to the higher branching ratio between the first two orders. Branching ratios in the first two order roots at our site were 18.6 for *F. mandshurica* and 10.4 for *L. gmelinii* (Wang et al. 2006). Therefore, as the predominant role of first order roots in the dynamic portion of the fine root pool, we conclude that the biomass turnover of first order roots should be carefully considered when assessing the C flows and nutrient cycles in forest ecosystem (Wells et al. 2002; Guo et al. 2008b).

Conclusion

In both *F. mandshurica* and *L. gmelinii*, first order roots always had shorter life spans, higher standing biomass, and greater biomass turnover than did second order roots. Compared with the branch-order based approach, the diameter-based approach underestimated biomass turnover in both species. The discrepancy between the two approaches was caused by the heterogeneity in the total root population. The branch-order-based method enabled separation of the heterogeneous root populations into relatively homogenous groups with different turnover rates. In contrast, the diameter-based approach failed to do so. Therefore, separating the fine root pool into different branch orders improves the accuracy of estimates for fine root turnover, and the understanding for belowground C allocation and nutrient cycling at the ecosystem level.

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